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Potential of Sweet Potato Mutant Lines for Bioethanol Production

Potensi Galur Mutan Ubi Jalar untuk Produksi Bioetanol

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ABSTRAK

Potential of Sweet Potato Mutant Lines for Bioethanol Production. Shoots of sweet potato Sari variety were irradiated at the doses of 0, 10, 20, 30 and 40 Gy. Irradiated shoots were planted and selected to obtain better mutant lines than that of the parent plant. Ten mutant lines were from the fourth generation which better morphology and productivity than that of the parent plant. The best productivity was found at mutant line number 40-2 which was 717.50 g/plant compared to parent plant with 622.50 g/plant. The highest glucose and starch content obtained were at the dose of 20 Gy which were 8.85 and 28.56 % respectively. The mutant line of Sari sweet potato has a potential to produce bio-ethanol. The bio-ethanol production from those of mutant lines at a range of 15.02 to 19.46 % compared to 13.67 % in the parent plant. The mutant line number 20 was the best line to produce bio-ethanol. The aim of this experiment was to find mutant lines having potential to produce bio-ethanol..

Key words : Sweet potato, mutant lines, production of bio-ethanol 13.67 %

ABSTRACT

Potensi Galur Mutan Ubi Jalar untuk Produksi Bioetanol. Telah diiradiasi tunas ubi jalar varietas Sari dengan dosis 0, 10, 20, 30 dan 40 Gy. Iradiasi tunas di tanam dan diseleksi untuk mendapatkan galur mutan yang lebih baik daripada tanaman induknya. Sepuluh galur mutan pada generasi keempat memperlihatkan morfologi dan produktivitas lebih baik dari induknya. Produktivitas terbaik diperoleh pada galur mutan nomor 40-2 dengan berat 717,50 g/tanaman dibanding tanaman induknya 622,50 g/tanaman. Kadar gula dan pati tertinggi diperoleh pada dosis 20 Gy yaitu masing-masing 8,85 dan 28,56 %. Galur mutan ubi jalar varietas sari berpotensi untuk produksi bioetanol. Produksi bioetanol berkisar antara 15,02 sampai 19,46 % dibanding tanaman induknya 13,67 %. Galur mutan nomor 20 adalah galur terbaik penghasil bioetanol. Tujuan penelitian ini adalah untuk mendapatkan galur mutan yang mempunayi potensi untuk produksi bioetanol.

Kata kunci : ubi jalar, galur mutan, produksi bioetanol

INTRODUCTION

Indonesian's oil production has been declining due to natural decline of oil reservoirs in production wells. On the other hand, the increasing demand for fossil fuel is triggered by the rapidly growth of population as shown in the last decade. This is a problem which has to be solved in order to full fill the fuel demand. Further the supply from other countries has been implied on rising cost. Based on those

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conditions, the government has disseminated a plan to reduce the dependency of Indonesia upon refined fuel by issuing the Presidential Regulation number 5, 2006 to sustain National Energy Policy to develop alternative energy sources as a substitution for refined oil. The Indonesian Government also issued the Presidential Regulation number 1, 2006 about providing and using bio ethanol as an alternative energy source [1]. To fulfill this target, superior crops for bio-ethanol production are needed. Based on the research of The Agency for Assessment and Application of Sciences and Technology (BPPT) it is acknowledged that Indonesia has 60 types of plants that have a potential to be used as source for bio-energy [2]. Cassava, corn, sugar cane and sweet potato are some of the plants available to be used as bio ethanol resources. Sweet potato has world-wide opportunity for producing bio-fuel, one attractive trait of this crop that it does not compete with other essential food crops.

Sweet potato (*Ipomoea batatas* L.) is one of the important tuber crops in Indonesia. This crop is used as an alternative source of carbohydrate, taking the fourth place after rice, corn and cassava. In Papua Province, sweet potato is used for staple food and for starch production in other Provinces [3]. The central production of sweet potato in Indonesia is West Java, Papua, and East Java provinces [4]. In 2008, the production of sweet potato from those provinces were 868.745 ton from 77.424 ha area [3]. However, this production has been decreased due to the decreasing of land area. For this reason, the production of useful mutant by gamma irradiation improving yield and quality of sweet potato is needed. According to Jusuf *et.al* [5], sweet potato breeding programs in Indonesia are directed to improve eating quality, disease resistance, yield and maturity.

One of the major approaches to improve sweet potato varieties are through vegetative propagation. Applications of nuclear technique in food and agriculture have contributed greatly in enhancing agriculture production of seed and vegetative propagated crops [6]. It has been shown that radiation has significant effects on the improvement of disease resistance and starch content. According to Wang *et.al* [7], irradiated shoot apices of sweet potato cultivar Kokei no. 14 by different doses resulted in significant increase in tuber yield from 218.8 g to 649.9 g in the mutant plant. Mutants with homogenous root skin color and high dry matter contents were obtained by irradiating cell suspension cultures with gamma rays [8]. The purpose of this experiment is to find the mutant lines from Sari variety of sweet potato which having higher production, starch content and potential to produce bio-ethanol.

MATERIALS AND METHODS

Plant Material

Sweet potato var. Sari used in this study is an important variety in Indonesia. Sari variety is red in root skin color and light-yellow in root flesh color, and is used as the parent plant for the mutants.

Gamma Irradiation

Young shoots around 200 shoots per dose were irradiated by gamma rays with the doses of 0; 10; 20; 30 and 40 Gy at the IRPASENA irradiator facility. Irradiated shoots were planted in the field and mutant lines were selected until the fourth generation. Yield evaluation trial was arranged in randomized complete block design with three replications. The plot size was 4 x 5 m with 0.25 x 1 m the distance between the plants. The planting area was in Bogor at an altitude about 200 to 300 m above sea level. Selection was based on morphology, productivity, carbohydrate content and bio ethanol production.

Carbohydrate content assay

The carbohydrate component i.e., starch glucose and amylose were analyzed in this study. Starch content was conducted based on NSI protocol where the tuber was

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chopped to small sizes then dialysis by 9.2 N hypo chloric acid and boiled at high temperature. The clear solution was obtained by filtering the liquid using whatman no. 2 filter paper. Clear solution was then reacted by 1 % anthrone solution and the occurring blue solution could be measured by Visible Spectrophotometer at wavelength 630 nm. Reducing sugar (glucose) content was also measured by using the same equipment without dialysis treatment. To obtain the amylose content in each sample, the samples were reacted with 0.2 % iodine solution, after that the absorbance of samples were measured by Visible Spectrophotometer at 630 nm.

Bioethanol production

Bio ethanol was produced from 5 kg fresh tubers which where mashed and boiled for one hour. A portion of alpha amylase was added (0.1 % per starch content) before cooking. The mashed tubers were cooled down and the pH adjusted to 4.2 by phosphoric acid. When the temperature in the liquefied mash had reached 60°C, the glucoamylase was added at the concentration of 0.06 % per starch content and the saccharification temperature was maintained at 60°C for one hour. Dry yeast and inorganic fertilizer were added after the mash cooling reach room temperature. The fermentation process to convert sugar to bio ethanol occurred during 64 hours by adding dry *Saccharomyces cereviceae*. Bio ethanol was separated from beer by using traditional distiller. The bio-ethanol product was then measured by Chromatography Gas.

RESULTS AND DISCUSSION

The morphological result of this research is given in Table 1, the data were taken 3.5 months after planting. The data display that no significantly effect of doses on leaves area of plants were shown, but length of plants were significantly effected by the doses of 30 and 40 Gy.

A major component of sweet potato storage root is starch. Table 2 presents the carbohydrate content and tubers weight of selected lines of sari sweet potato.

Table 1. Morphology and number of mutant at fourth generation of sari sweet potato

Doses (Gy)	Length of plant (cm)	Leaves area (cm^2)	Number of tuber/plant	Form of tuber	Number of selected lines
Original plant	98.39	44.50	2	Round	-
10	86.20	45.02	3	Round	3
20	86.05	44.30	2	Round	1
30	78.45	44.00	3	Long	2
40	72.04	45.21	3	Long to round	4

The largest tuber size was found at mutant line number 40b to be 717.50 g/plant, however, this line has no different glucose content than that of the original plant. Contrastingly, the weight of mutant line number 20a was 360 g/plant but compared to other lines and that of the original plant, this line showed that the carbohydrate content within glucose, starch and amylose has the highest content compared to the other lines. The concentration of genes controlling starch content in a cultivar is essential for the development of cultivars with high starch content (10). Carrol (11) reported that mutations in sweet potato might arise from genetic instability. These events may be responsible for chromosome breakage, alteration of DNA methylation, single base changes, and changes in copy number of repeated sequence. According to Nakatani (12), the biochemical activities of starch synthesis are also factors determining the starch content. Starch is synthesized by starch synthase, and ADP-glucose is

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Table 2. Carbohydrate content of mutant lines and original plant of sari sweet potato

Mutant lines	Tuber weight/plant (g)	Starch (%)	Amylose (%)	Glucose (%)
Original plant	622.50d	22.78a	13.05a	7.09d
10-1	525.00c	23.43ab	13.15a	6.92d
10-2	456.25b	25.7cd	14.34abc	5.46a
10-3	460.88b	25.09bcd	14.43abc	8.51e
20-1	360.00a	28.56ef	15.97d	8.85e
30-1	659.34e	24.57abc	14.08abc	6.33bc
30-2	704.44g	26.89de	14.39abc	6.10b
40-1	679.92f	28.26ef	14.74bcd	8.80e
40-2	717.50g	23.66ab	13.57ab	6.15b
40-3	621.75d	28.89f	15.17cd	5.84ab
40-4	715.00g	28.39ef	14.99bcd	6.78cd

Note : Values followed by the same letter within a column do not differ significantly at 5 %.

predominant glucose donor in the root of sweet potato. It could be assumed that the dose of 20 Gy could effect on the pathway of carbohydrate of sweet potato, however, this dose has very low frequency mutation than the other dose (Table 1). The better dose resulting in highest starch content was obtained at 40 Gy, where the highest starch content is 28.89 % found at mutant line number 40c.

To produce bio-ethanol, glucose content is a very important key for fermentation, from the data shown the original plant has 7.09 % glucose which was higher than some mutant lines. However, some mutant lines have higher amylose and starch content than that of the original plant, furthermore they have higher bio-ethanol production than that of the original plant. The highest glucose content was obtained by mutant line number 40c with concentration of 28.89 %. Amylose content also was increased by mutation, the data showed the highest amylose content was found at mutant line number 20a was 2.47 % higher than that of the original plant. The result of this study is similar to Ooe's *et.al* result [13], they reported that amylose content and morphology of sweet potato mutant lines varied by variation of doses. Sudarmonowati *et.al* [14] reported, the amylose content of cassava mutant line of Darulhuda variety was higher than that of the original plant, the increase of amylose content was 3.33 % by gamma irradiation at the dose of 300 Gy.

To obtain the optimum time for fermentation, two kind of yeast were conducted toward sweet potato cv. SARI. Table 1 was shown that the optimum time to produce bio ethanol from two kind of yeast were 64 hours, in comparing between dry yeast and wet Haken no.1 treatment, the ethanol production by using dry yeast was 8.2 % meanwhile wet Haken No.1 treatment was only 7.9 %. According to Atthasampunna *et.al* [15], fermentation of cassava by using wet Haken no.1 could produce bio-ethanol around 8.6 to 9.5 % at high temperature cooking and 8.5 to 9.3 % bio-ethanol at low-temperature cooking.

Typical fermentation processes for the dry and wet Haken No. 1 yeast are shown in Table 3. Both processes showed a similar decrease in total sugars during the production of ethanol and yeast cell mass. At the end of 72 h, total residual sugar remind about 0.57 and 0.43 % by both yeasts, meanwhile at the beginning of fermentation the total sugar and starch were 13.15 % and 7.24 % respectively.

Yeast can work well when the total sugar content is around 16 %, on the other hand, if the total sugar from digestion of starch, amylose and sugar content were too high, the mash should be dilluted by water to obtain the cells which could survive and

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produce ethanol. From this data the actually ethanol product at the end of fermentation were 13.13 and 13.67 % by wet Haken No. 1 and dry yeast treatments respectively (Table 3).

mutant line number 20a to be 19.46 % compared to the original plant only 13.67 %. This line is more potential to produce bio-ethanol than the other mutant lines of sweet potato. In the previous study, the mutant

Table 3. The condition of original sweet potato fermentation at two kind yeast treatments

Time (hr)	Temperature of fermentation (^{0}c)	pH	TS (%)	RS (%)	Ethanol (%)	Acidity	Amount of cell	Viscosity
0 – H	33	5.39	13.30	6.37	-	-	-	-
D	33	5.65	13.15	7.24	-	-	-	-
16 – H	32	4.97	5.21	1.88	4.7	4.3	15.56	200
D	32	5.02	4.92	2.02	3.5	3.5	4.75	300
40 – H	31	4.84	0.84	0.43	7.9	3.8	15.12	175
D	32	5.02	0.57	0.87	8.2	2.8	6.75	250
64 – H	31	4.87	0.72	0.65	7.9	2.3	10	75
D	31	5.02	0.57	0.43	8.2	2.3	6.25	200

Note : H: wet Haken No.1 yeast; D :Dry yeast, TS : Total sugar; RS : Reducing sugar

Table 3 was a display from two kind of yeast treatment, showing dry yeast was better than wet Haken No.1 yeast treatment, and this yeast could produce higher ethanol than wet Haken yeast at 40 hour fermentation. The maximum time for fermentation was found at around 64 hour. Furthermore, the data of Table 3, for fermentation of tubers of sweet potato mutant lines dry yeast *Saccharomyces cereviceae* was used.

Figure 1 was the bio-ethanol produced by each of sweet potato mutant lines. The highest concentration was found at the

lines Shiroyutaka variety of sweet potato produced 23.52 % bio-ethanol (16). It is possible that mutant lines of sweet potato var. Shiroyutaka contain amylose, glucose and starch higher than Sari sweet potato mutant lines.

The production of bio-ethanol significantly effect by carbohydrate content of sweet potato, it related to data in Table 2. The ratio of production of bio-ethanol from sweet potato depends on the carbohydrate content. Glucose can be directly converted to bio-ethanol, starch and amylose if digested by enzymes to glucose. The ratio of

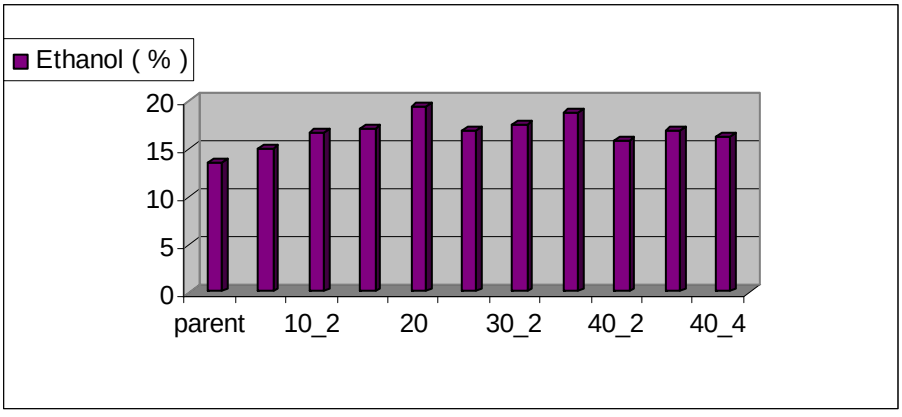

Figure 1. Production of bio-ethanol from mutant lines and original plant

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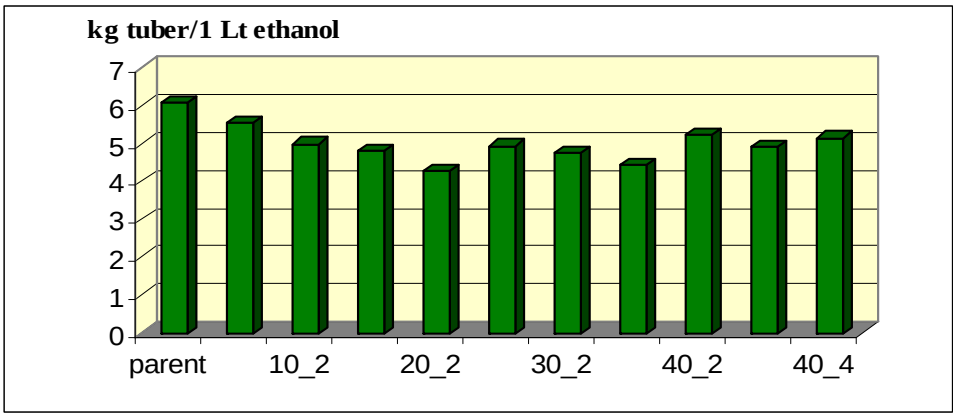

Figure 2. Consumption of mutant lines and parent plant (kg) to produce 1 liter of bio-ethanol

sweet potato mutant lines to produce 1 liter of bio-ethanol is displayed in Figure 2.

In comparison with Figure 1, the highest bio-ethanol concentration was found in mutant line number 20a. The highest of glucose, amylose and starch content will result in highest bio-ethanol production too, on the other hand, low production of ethanol in mutant lines is that from mutant lines with low glucose, amylose and starch content. From Figure 2, it is found that the lowest tuber need to produce bio-ethanol was 4.28 kg is of mutant line number 20a compared to the original plant which needed 6.09 kg tuber for the same purpose. The mutant lines number 30b and 40d have also lower tuber need than original plant i.e. 4 and 4.12 kg per 1 liter bio-ethanol. The ratio of tubers to bio-ethanol is very important to calculate how much bio-ethanol can be produced from 1 ha land area. Some researches reported the ratio of sweet potato to bi-ethanol was about 7 to 7.5 kg per 1 liter bio-ethanol, this is only based on the starch content of sample about 15 to 20 %, no. calculation of glucose and amylose content was carried out. According to Nurdyastuti [17], the conversion of starchy resources to bio-ethanol production varied from cassava to sweet potato. To produce 1 liter of bio-ethanol 8 kg of sweet potato is needed compared to cassava which only need 6.5 kg, on the other hand, the ratio of sweet potato to produce bio-ethanol is larger than that of cassava. Comparing between carbohydrate component of cassava and sweet potato, cassava has no glucose contain like sweet potato. Actually, the sweetness of sweet potato is very important key beside starch and amylose to produce bioethanol.

CONCLUSION

From the result of this research it can be concluded that mutation by the doses of 10 to 40 Gy can has an effect on tuber size and carbohydrate content. The highest potential to produce bio-ethanol was found at mutant lines number 20a to be 19.46 %, this line also has the smallest ratio than that of the original plant to produce bio-ethanol from sweet potato.

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